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ANTI-CANCER ACTIVITY OF THE EXTRACTS OF *EUGENIA JAMBOLANA*

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ABSTRACT

Cancer, after cardiovascular disease, is the second leading cause of death. Worldwide about 10 million people per year are diagnosed with cancer and more than 6 million die of the disease and over 22 million people in the world are cancer patients. It is a complex disease that is associated with a wide range of effects both at the molecular and cellular levels. Natural protection against cancer has recently been receiving a great deal of attention not only from cancer patients but also from physicians as well. In this study the effect of extracts from various parts of the fruit of *Eugenia jambolana* (Black Plum) is observed on breast cancer and prostate cancer cell lines.

KEYWORDS:*Eugenia jambolana*, **Prostate cancer, cell viability, apoptosis and cancer cell lines**

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INTRODUCTION

Ayurveda is a system of medicine that originated in India several thousand years ago. The term Ayurveda combines two Sanskrit words: *ayur*, which means life, and *veda*, which means science or knowledge. Ayurveda is "the science of life."[1]. Reverse Pharmacology on the selected plants may lead not only to new leads and drug candidates but also to novel targets and pharmacodynamic efforts. For example, investigations on *Coleus forskohlii,* used as pickles in some parts of India, led to isolation of forskolin[3-6], with multifaceted effects. The latter were mediated by activation of adenylate cylase and increased concentration of cyclic adenosine monophosphate (cAMP)[7-9]. Subsequently, forskolin has served as a very important tool in molecular pharmacology and endocrinology[10-14]. Prostate cancer is the third most common cause of death from cancer in men of all ages and is the most common cause of death from cancer in men over age 75. Prostate cancer is rarely found in men younger than 40.[15] In our present study, we have investigated the effect of extracts from various parts of the fruit of *Eugenia jambolana* (Black Plum) is observed on breast cancer and prostate cancer cell lines. We have examined the apoptosis and cell viability of prostate and breast cancer cell lines upon the induction with the extracts of *Eugenia jambolana*

MATERIALS AND METHODS

Preparation of Seed Extracts

20gms of powdered seeds were prepared for Soxhlet extraction. 100ml of Hexane and ethyl acetate mixture (1:1) was taken as Solvent to run the extraction process (Fuleki and Francis, 1968). The extraction was allowed to run till the solution in the equipment empties into the round bottom flask. The extract is transferred to a 500-ml boiling flask. Residual acetone/chloroform is removed in a rotary evaporator at 40°C under vacuum. Remaining aqueous extract is made up to a known volume (usually 100 ml) with acidified deionized distilled water. If the sample is to be analyzed within 2 days, it is stored at 4°C.

Preparation of Peel Extracts

5gms of powdered peel was prepared for Soxhlet extraction. 100ml of Hexane and ethyl acetate mixture (1:1) was taken as Solvent to run the extraction process (Oszmianski and Lee 1990). The extraction was allowed to run till the solution in the equipment empties into the round bottom flask. The extract is transferred to a 500-ml boiling flask. Residual acetone/chloroform is removed in a rotary evaporator at 40°C under vacuum (Wrolstad and Durst 1998). Remaining aqueous extract is made up to a known volume (usually 100 ml) with acidified deionized distilled water. If the sample is to be analyzed within 2 days, it is stored at 4°C.

Preparation of Pulp Extract

50 g plant material was mixed with 1:1 (w/v) acetone using a general-purpose homogenizer (Strack and Wray, 1994; Jackman and Smith, 1996). The extract (filtrate) is separated from the insoluble plant material by filtering the slurry through a Whatman no. 1 filter paper by vacuum suction using a Buchner funnel. The plant material is reextracted with 70% (v/v) aqueous acetone until a clear or faintly colored solution is obtained. The plant material pH is adjusted with aqueous acidified acetone, if plant material has a pH ≥4. Filtrates were pooled and plant material was discarded. (Strack and Wray 1989). The filtrate was transferred to a separating funnel, 2 volumes of chloroform was added and gently shaken by turning the funnel upside down a few times. The sample was stored overnight at 4°C or until a clear partition between the two phases is obtained. The aqueous phase (upper portion) is transferred to a 500ml boiling flask and the residual acetone/ chloroform was removed in a rotary evaporator at 40°C under

vacuum. The remaining aqueous extract was made up to a known volume with acidified deionized distilled water, water, methanol, or other appropriate solvent. The solvent is stored at 4°C, if it is to be analyzed within 2days.

Annexin-V and propidium iodide staining Procedure

The 1X 10^6 single cells were washed in 2X cold PBS. The cells were again washed with 2X Binding Buffer. The cells were re-suspended in 100ul Binding Buffer and Annexin V-FITC. (Titer from 0.1μg – 1.0μg). They were incubated at room temperature for 10 minutes. 400μl Binding Buffer was added containing 1μl PI. They were incubated on ice for 15 minutes and analyzed by flow cytometry within one hour.

BrdU assay for cell proliferation

The cells were cultured in a 96-well microtiterplate and are pulse-labeling them with BrdU. Only the proliferating cells incorporate BrdU into their DNA. The cells were fixed with FixDenat solution. The FixDenat solution denatures the genomic DNA, exposing the incorporated BrdU to immunodetection. The BrdU label was located in the DNA with a peroxidase-conjugated anti-BrdU antibody (anti-BrdU-POD). The bound anti-BrdU-POD was quantified with a peroxidase substrate.

RESULTS AND DISCUSSION

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other appropriate solvent. The solvent is stored at 4°C, if it is to be analyzed within 2days.

Effect of different extracts of Eugenia jambolana on the apoptosis of MCF-7 and PC3 cells

MCF-7 (breast cancer cell line) and PC3 (prostate cancer cell line) were cultured in DMEM serum free medium for 24 hours. During the culturing the effect of the above extracts was checked using flow cytometry.

The effect of *Eugenia jambolana* on cancer cell-lines was investigated using flow cytometry. The cells were revived and incubated with Propidium Iodide and Annexin-V. After incubation for 10 minutes on ice, the cells were subjected to analysis with BD-FACScan. The results were shown in Figures 1 and 2.

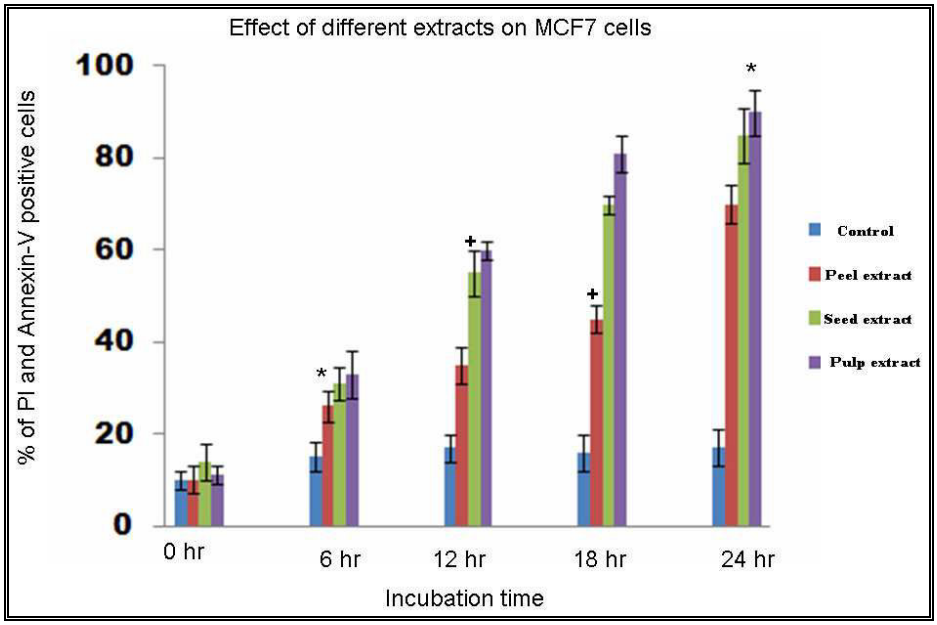

Figure 1.
Effect of various extracts of Eugenia jambolana leading to apoptosis of MCF-7 cells. Mean error bars in the graph represent the mean ± standard error from the duplicate samples that were tested.

Significance of difference: * represents P<0.01, students t test and + represents p<0.05.

The above results clearly show that the effect of seed and pulp extracts on the apoptosis of breast cancer cell line MCF-7 cells is more than the effect of peel extract. Hence, we proceeded to further analysis with pulp and seed extracts of *Eugenia jambolana.*

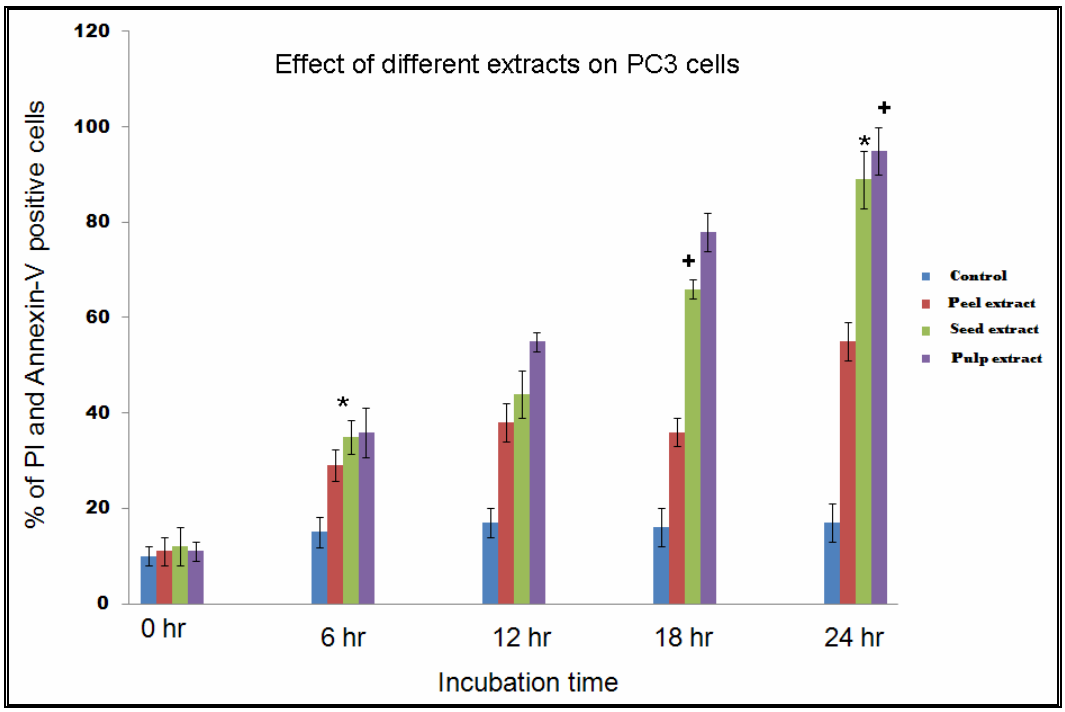

Figure 2.
Effect of various extracts of Eugenia jambolana leading to apoptosis of MCF-7 cells. Mean error bars in the graph represent the mean ± standard error from the duplicate samples that were tested.

Significance of difference: * represents P<0.01, students t test and + represents p<0.05.

The above results clearly show that the effect of seed and pulp extracts on the apoptosis of prostate cancer cell line PC3 cells is more than the effect of peel extract. Hence, we proceeded to further analysis with pulp and seed extracts of *Eugenia jambolana.*

Analysis of cell proliferation using BrdU assay

Proliferation of MCF-7 and PC3 cells were analyzed using BrdU assay in presence and absence of pulp extracts of *Eugenia jambolana*. The results were shown in Figures 3 and 4.

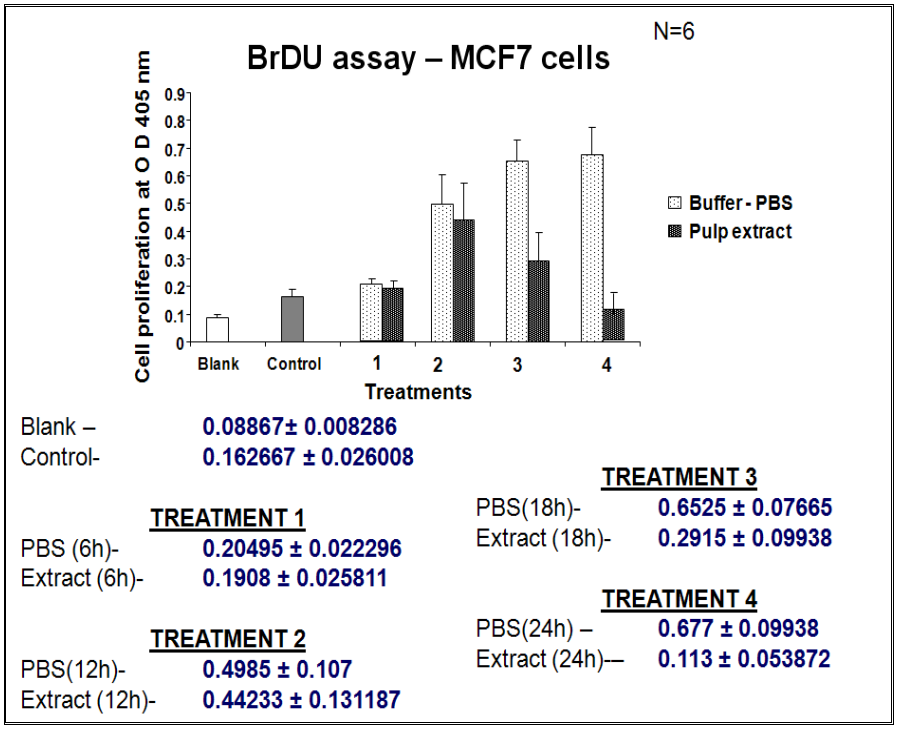

Figure 3.

Effect of pulp extracts of Eugenia jambolana on cell proliferation of MCF-7 cells

Figure 1. Effect of various extracts of Eugenia jambolana leading to apoptosis of MCF-7 cells. Mean error bars in the graph represent the mean ± standard error from the duplicate samples that were tested.

Significance of difference: P<0.05, students t test

The above results clearly show that the effect of pulp extracts on the cell proliferation. Here it is shown that the cell proliferation of MCF-7 cells has been inhibited by pulp extract of *Eugenia jambolana*.

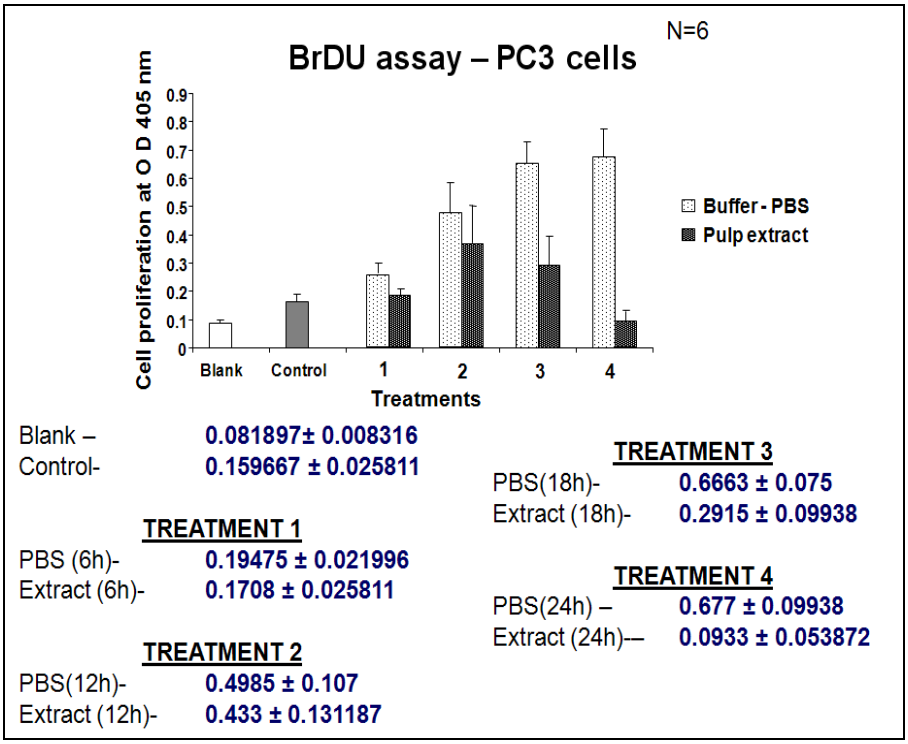

Figure 4.
Effect of pulp extracts of Eugenia jambolana on cell proliferation of PC3 cells Mean error bars in the graph represent the mean ± standard error from the duplicate samples that were tested.

Significance of difference: P<0.05, students t test

The above results clearly show that the effect of pulp extracts on the cell proliferation. Here it is shown that the cell proliferation of PC-3 cells has been inhibited by pulp extract of *Eugenia jambolana*.

CONCLUSIONS

The objective of the project is to study the anti-cancerous activity of extracts of various parts of the fruit of *Eugenia jambolana* that may be useful in fighting cancer, mostly concentrating on Breast and Prostate cancers. The extracts from the three parts of the fruit (Pulp, seed and peel) were observed to induce apoptosis of the cell lines, reduce colony formation of the tumor cells and inhibit the cell proliferation when applied to the MCF-7 (breast cancer cell line) and PC3 (prostate cancer cell line). It was observed that the Pulp extracts were more effective in inducing apoptosis, reducing colony formation and inhibiting the cell proliferation of both MCF-7 and PC3 cell lines, followed by the Seed extracts that induced apoptosis of the cell lines more effectively than reducing colony formation and inhibiting cell proliferation in comparison with Pulp extracts. Whereas the Peel extracts were seen to have very less effect in inducing apoptosis, reducing colony formation and inhibiting the cell proliferation of both MCF-7 and PC3 cell lines in comparison with both Pulp and Seed extracts.

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